Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5326–5330, June 1992
Physiology

Parathyroid hormone-related protein in the rat urinary bladder: A smooth muscle relaxant produced locally in response to mechanical stretch

MICHIKO YAMAMOTO, SANDRA C. HARM, WILLIAM A. GRASSER*, AND MARK A. THIEDE*†

Department of Bone Biology and Osteoporosis Research, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

Communicated by Hector F. DeLuca, February 28, 1992 (received for review October 25, 1991)

ABSTRACT **Parathyroid hormone-related protein (PTHrP) gene expression in the pregnant rat uterus has been shown to be dependent on occupancy of the uterus by the fetus. To further test the hypothesis that the synthesis of PTHrP in smooth muscle tissue is regulated by mechanical stretch, we conducted experiments using the rat urinary bladder as a model of an expansible hollow organ. The results indicate that PTHrP mRNA levels do change in response to the stretch of the bladder wall. Under normal conditions PTHrP mRNA levels in the bladder correlated with the urine volume—namely, the extent of bladder distension. When bladders were maintained empty *in vivo*, PTHrP mRNA levels decreased gradually. Conversely, when bladders were distended by the accumulation of urine, levels of PTHrP mRNA increased dramatically with time. When distension was limited to one-half of the bladder, the increase in PTHrP mRNA was observed only in the distended portion. Histochemical studies performed on distended bladder tissue indicated the presence of PTHrP immunoreactivity in smooth muscle cells. Isolated organ bath studies were used to examine the possible physiological role of PTHrP in smooth muscle tonicity. *In vitro* responsiveness of bladder muscle strips to exogenous PTHrP was dependent on the *in vivo* condition of the bladder. In muscle strips obtained from bladders kept empty *in vivo*, PTHrP-(1–34)-NH_2 relaxed carbachol-induced contraction in a dose-dependent manner but failed to relax the contraction in muscle strips from distended bladders that had high endogenous PTHrP expression. These results and the previous findings in the rat uterus suggest a physiological role of PTHrP in bladder smooth muscle function.**

Parathyroid hormone-related protein (PTHrP) was originally identified in tumors associated with the syndrome of humoral hypercalcemia of malignancy (1–3). Synthetic N-terminal fragments of PTHrP were shown to have biological effects very similar to those of parathyroid hormone (4–9). Subsequently, it has been shown that, unlike the parathyroid hormone gene, the expression of which is confined to the parathyroid gland, the PTHrP gene is expressed in a wide spectrum of normal mammalian and avian tissues (10–18). Although little is known about the physiological function of PTHrP in normal tissues, widespread tissue distribution of this peptide suggests that PTHrP may have a paracrine or autocrine role (6).

It is of interest that PTHrP immunoreactivity is detected consistently in smooth muscle cells of vascular and nonvascular origin (19). In support of a physiological role for PTHrP in smooth muscle function, previous studies have shown that exogenous PTHrP-(1–34) can inhibit contractile activity of vascular and nonvascular smooth muscle (18, 20–23). Furthermore, specific binding for PTHrP-(1–34) has been demonstrated in vascular smooth muscle cells (24). Recent studies in the uterus of pregnant rats indicate that the expression of the PTHrP gene in the myometrium during late gestation is controlled by intrauterine occupancy by the fetoplacental unit (25). This observation indicates that a unique mechanism regulates the production of PTHrP in relation to smooth muscle function.

We designed the present study to test (*i*) whether the production of PTHrP by smooth muscle is regulated by mechanical stretch and (*ii*) whether PTHrP produced locally functions in the regulation of smooth muscle tonicity. For these purposes, we chose the rat urinary bladder as a model organ for the following reasons. (*i*) In preliminary Northern blot studies, we found that PTHrP mRNA is present in this organ. (*ii*) The urinary bladder is a flexible smooth muscle chamber that repeats expansion and contraction periodically under physiological conditions. Its structure and function are simple compared to other hollow muscular organs such as the gastrointestinal tract or uterus.

We report here that PTHrP mRNA expression in the rat urinary bladder is functionally regulated by a local mechanism related to bladder-wall distension and that exogenous PTHrP-(1–34) induces relaxation in bladder smooth muscle. The findings support the view that the synthesis of PTHrP is regulated by stretching and that PTHrP produced locally in turn regulates muscle tonicity in the smooth muscle tissue.

MATERIALS AND METHODS

Animals and *in Vivo* Treatment. Experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of our company. Adult Sprague–Dawley rats (both sexes) weighing 250–350 g (Taconic Farms) were used. After *in vivo* treatment (described below), bladders were removed and used for PTHrP mRNA measurement, organ bath studies, or immunohistochemistry.

Study of normal conditions (no treatment). Rats were anesthetized by i.p. injection of ketamine (Aveco, Fort Dodge, IA; 75 mg/kg), content (urine) volume was measured for each bladder, and bladder samples were pooled according to urine volume.

Time course study. Under pentobarbital anesthesia (initial dose, 50 mg/kg i.p., with addition of smaller amount when necessary), bladders were emptied by aspiration of urine with a 27-gauge needle. Control bladders were obtained immediately after anesthesia. Animals were divided into two groups. In group 1 rats, bladders were maintained empty by cutting the ureters and draining urine externally onto gauze. In some rats, bladders were kept empty by a different method,

Abbreviation: PTHrP, parathyroid hormone-related protein.

*Present address: Osteoporosis Group, Pfizer Central Research, Groton, CT 06340.

†To whom reprint requests should be addressed at: Osteoporosis Group, Box 266, Pfizer Central Research, Eastern Point Road, Groton, CT 06340.

continuous drainage of urine by a urethral catheter. In group 2 rats, bladders were gradually distended by accumulation of urine. During the experiments, both groups of rats were administered physiological saline (1–2 ml every 2 h) subcutaneously to yield an adequate urine flow. Bladder specimens were obtained at 2-h intervals for 6 h. The animals that showed micturition reflex (outflow of urine from the urethra) during the treatment were excluded from the analysis. No urethral ligature was applied to prevent urine leakage in this study, because in the pilot studies we found that the majority of rats did not show micturition reflex when an adequate depth of anesthesia was maintained. With this method, we could exclude the influence of the micturition reflex on the results and avoid unnecessary stimuli to the urethral area.

Localized distension of the bladder. After pentobarbital anesthesia and emptying of the bladder as described above, either the distal or proximal half of the bladder was covered with elastic tubing (inner diameter, 4 mm; length, 5 mm) to prevent distension in this portion and the other half was left uncovered to allow distension by urine accumulation. To avoid obstructing the blood flow in the bladder, no ligature was used for covering the proximal half; only a loose ligature was made to fix the tubing around the distal half of the bladder. Three to 4 h later, bladders were cut horizontally in half, and the distended and nondistended halves were pooled separately. Control bladders (kept empty or distended for 4 h) were prepared in the same way as described in the time course study and were cut horizontally in half.

Northern Blot Analysis. RNA was prepared from pools of frozen bladders as reported (10). Total RNA (20 μg per lane) was separated by electrophoresis in a 1% formaldehyde/agarose gel and transferred to a nitrocellulose filter. After prehybridization, the filter was hybridized for 48 h at 42°C to a ^{32}P-labeled 712-base-pair *Pvu* II–*Xba* I fragment of rat PTHrP cDNA (10, 25). Subsequently, the same filter was reprobed with a chicken β-actin cDNA (Oncor, Gaithersburg, MD). After hybridization, filters were exposed to Kodak XAR film for 2–3 days with intensifying screens.

Immunohistochemistry. After a 6-h distension *in vivo*, a bladder sample was formalin-fixed, paraffin-embedded, and sectioned. PTHrP immunoreactivity was detected by peroxidase-antiperoxidase technique using an immunochemistry kit (Oncogene Science, Manhasset, NY) that utilized PTHrP-specific monoclonal antibody raised against recombinant PTHrP-(1–141) (19). The epitope has not been determined by the manufacturer. Confirmation of specific staining was obtained using control monoclonal antibody (no cross-reactivity with PTHrP) provided with the kit.

Organ Bath Studies. Prior to organ bath studies, bladders were kept empty or distended *in vivo* for 2–4 h to control the bladder condition. Immediately after removal of the bladder from the animal, a longitudinal muscle strip ($\approx$15 × 2 mm) was cut from each bladder. Isolated strips were placed in the organ bath containing 50 ml of Van Dyke–Hastings buffer (110 mM NaCl/30 mM $NaHCO_3$/0.8 mM NaH_2PO_4/0.2 mM Na_2HPO_4/6.0 mM KCl/0.5 mM $MgCl_2$/0.5 mM $CaCl_2$/2.8 mM glucose). The buffer was maintained at 37°C and continuously oxygenated with 95% O_2/5% CO_2. Resting tension (0.5 g) was applied to each strip and the change of tension was measured isometrically with a force transducer (Narco myograph, Houston) and recorded on a physiograph (Narco Mark IV). The experiments were started after a baseline stabilization period of 30 min. In the first control stimulation, carbachol (final concentration, 0.35 μM) was added to the organ bath and contraction was measured. After a 30-min equilibration in fresh buffer, PTHrP-(1–34)-NH_2 (final concentration, 2.2–220 nM) was added to the organ bath followed by carbachol (0.35 μM). After measuring contraction, the chamber was again washed with fresh buffer. After 30 min, the muscle strips were again challenged with carbachol alone (second control stimulation) to confirm that the contractility of the strips had not changed during the 2-h experimental period.

PTHrP-induced relaxation was estimated as a change of muscle tension from the maximal point of carbachol stimulation. Data are expressed as mg of tension developed per mg of tissue (mean ± SEM). Statistical significance was evaluated by analysis of variance and post hoc contrast test (Tukey's honest significant difference) using the statistical software package for Macintosh computers. In the dose–response study, the muscle tension induced by carbachol in the presence of PTHrP-(1–34)-NH_2 was expressed as a percentage of the maximal contraction induced by carbachol alone. The EC_{50} value was determined by the least square linear regression analysis.

Materials. PTHrP-(1–34)-NH_2 was synthesized by a solid-phase method and purified by HPLC as described (26). The purity of the synthetic peptide was >98%, determined with data from amino acid sequencing and analytical reverse-phase HPLC. All other drugs and reagents were purchased from commercially available sources.

RESULTS

Correlation of PTHrP mRNA Levels with Bladder-Wall Distension. Under normal conditions (without any treatment), the urine volume inside the bladder ranged from 0.1 to 3.0 ml, and PTHrP mRNA levels in the individual bladders varied substantially. When bladders were grouped according to urine volume as shown in Fig. 1, PTHrP mRNA expression was higher in markedly distended bladders than in less distended ones. Bladders that were only slightly filled (urine volume, <0.3 ml) were excluded from this particular analysis because we assumed that these bladders might have been recently emptied after full distension. In such bladders, levels of PTHrP mRNA might reflect the effect of distension prior to emptying.

To further investigate the effects of bladder-wall distension on PTHrP mRNA levels and to examine the time course of changes in PTHrP mRNA expression, we kept the bladder in an empty or distended state *in vivo*. When bladders were maintained empty by cutting the ureters, PTHrP mRNA levels decreased with time (Fig. 2). A similar time course of decrease in PTHrP mRNA expression was observed in

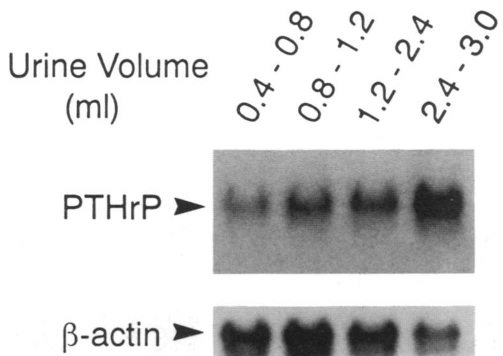

FIG. 1. Comparison of PTHrP mRNA expression among bladders with different volumes of urine. Rats were anesthetized without any pretreatment. Before removing the bladder, urine was aspirated with a fine needle (27 gauge) and the volume was measured. Bladders were grouped (n = 4–10 for each group) according to the urine volume as indicated. Two bladders from each group were randomly selected for analysis. PTHrP mRNA levels were determined by Northern blot analysis with a rat PTHrP cDNA probe (10, 25). Equal loading of total RNA (20 μg per lane) was monitored by ethidium bromide staining of the gel. The filter was reprobed with chicken β-actin cDNA. The findings were reproduced in three experiments.

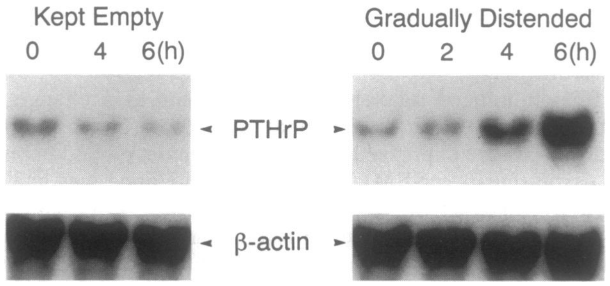

FIG. 2. Time course of changes in PTHrP mRNA levels in bladders kept empty or distended *in vivo*. PTHrP and β-actin mRNAs were determined by Northern blot analysis and the reproducibility was confirmed as described in Fig. 1. After being emptied of residual urine by aspiration with a 27-gauge needle, bladders were kept empty by cutting the ureters (*Left*) or were distended gradually by accumulation of urine (*Right*). Bladder specimens ($n = 2$) were obtained at indicated times (in hours) after treatment. Control bladders (indicated as 0 h) were obtained immediately after anesthesia. Autoradiographic exposures were adjusted for each set of experiments to allow easy visualization to 5 days (*Upper Left*) and to 3 days exposure (*Upper Right*).

bladders kept empty by continuous drainage of urine by urethral catheter (data not shown). In contrast, when bladders were gradually distended by the accumulation of urine, PTHrP mRNA levels increased dramatically with time (Fig. 2). The maximal urine volume retained in the bladder during 6 h of distension was 2.5 ml. Thus we assumed that the speed and the magnitude of bladder distension in this study were well within the physiological range.

We next examined the effect of local stretching on PTHrP mRNA expression. When either the proximal or distal half of the bladder was selectively distended, PTHrP mRNA expression was much higher in the distended half than in the nondistended half (Fig. 3). In contrast, there was no difference in PTHrP mRNA levels between the proximal half and the distal half of the control bladders, in empty and distended states (Fig. 3).

Immunohistochemistry. In tissue sections obtained from a bladder distended for 6 h, smooth muscle cells were stained positively with an anti-PTHrP monoclonal antibody (Fig. 4*A*). The specificity of PTHrP immunoreactivity was confirmed by negative staining with control monoclonal antibody (Fig. 4*B*).

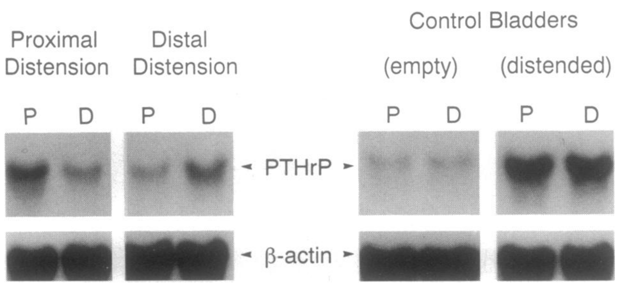

FIG. 3. Effects of local distension on PTHrP mRNA levels in the bladder. (*Left*) After emptying the bladder by aspiration of residual urine, either the proximal (P) or distal (D) half of the bladder was selectively distended by applying a small tubing (inner diameter, 4 mm; length, 5 mm) around the other half. Three to 4 h later, the bladders ($n = 4$) were cut horizontally in half and PTHrP mRNA levels were compared between distended and nondistended halves. Similar results were obtained in a separate set of experiments. (*Right*) Control bladders ($n = 4$) kept empty or distended for 4 h *in vivo* (treated in the same way as described in the legend for Fig. 2) were cut horizontally in half and PTHrP mRNA levels were compared between the proximal and distal halves.

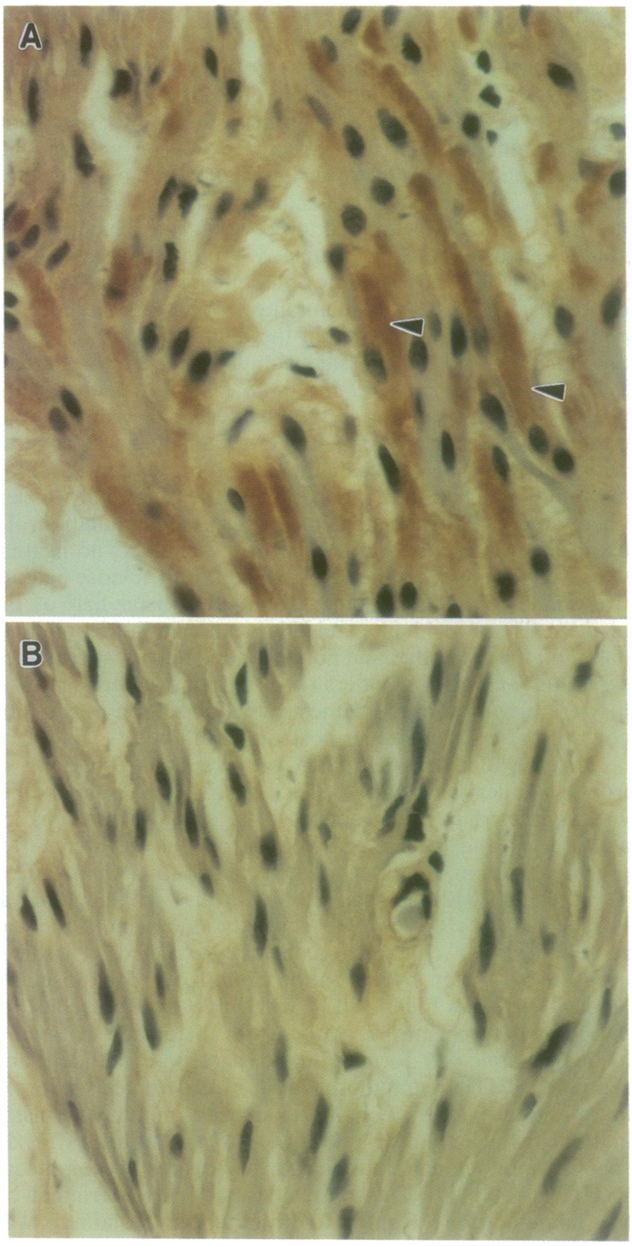

FIG. 4. Immunoreactive PTHrP in smooth muscle cells of rat urinary bladder. Paraffin-embedded tissue sections were prepared from a bladder distended for 6 h *in vivo* and were stained with anti-PTHrP monoclonal antibody (*A*) or control antibody (*B*). Brown staining (shown by arrowhead) indicates positive immunoreactivity. Counter staining was obtained with hematoxylin. (×200.)

Muscle-Relaxing Effects of Exogenous PTHrP-(1–34)-NH_2. To address a possible role for PTHrP in the regulation of muscle tonicity, we examined the ability of exogenous PTHrP-(1–34)-NH_2 to inhibit carbachol-induced contraction in bladder smooth muscle *in vitro*. In muscle strips from bladders kept empty for 2 h, PTHrP-(1–34)-NH_2 (2.2–220 nM) relaxed the carbachol-induced contraction in a dose-dependent manner (data not shown) with an EC_{50} value in the nanomolar range, a result similar to previous studies on other smooth muscle tissues (18, 20–23). The specificity of muscle-relaxing effects of PTHrP-(1–34)-NH_2 had been confirmed in other tissue (18) and in the rat urinary bladder (data not shown) by immunoneutralization experiments with rabbit antiserum raised against PTHrP-(1–34)-NH_2 and shown to specifically bind PTHrP-(1–34)-NH_2 (27). Interestingly, the muscle-relaxing effects of exogenous PTHrP were dependent on the condition of bladder maintained *in vivo*. When the effects of 44 nM PTHrP-(1–34)-NH_2, which induced near maximal relaxation in the dose–response study, were compared with control bladders and bladders kept empty for 2 h and 4 h, the magnitude of muscle relaxation was greater in bladders kept empty for longer period, although the differences were not statistically significant (data not shown). The difference in PTHrP-(1–34)-NH_2 effects was more clearly shown between empty and distended bladders. As shown in Fig. 5 *Left*, 44 nM PTHrP-(1–34)-NH_2 reduced the carbachol-induced tension by ≈50% in muscle strips from bladders that had been kept empty for 4 h. In contrast, when bladders had been distended for 4 h prior to tissue bath experiments, the same dose of PTHrP-(1–34)-NH_2 failed to relax the carbachol-induced contraction (Fig. 5 *Right*).

DISCUSSION

Expression of PTHrP mRNA has been reported in a variety of normal mammalian and avian tissues (10–18), although its presence in the urinary bladder has yet to be reported. In a recent tissue survey, we have found relatively high levels of PTHrP mRNA in the rat urinary bladder, supporting the results of immunohistochemical studies that demonstrated PTHrP-specific immunoreactivity in the human bladder (19). However, the detection of PTHrP mRNA or PTHrP immunoreactivity in certain tissues or organs does not necessarily implicate physiological role(s) for PTHrP in these sites. Therefore, to obtain insight into the possible physiological role of PTHrP in the urinary bladder, we examined the expression of the PTHrP gene in relation to bladder function.

Our hypothesis that the expression of the PTHrP gene in smooth muscle tissue is regulated by mechanical stretch was based on previous findings in the pregnant rat uterus that showed that the synthesis of PTHrP correlated with intrauterine occupancy (25). To test this hypothesis in the rat urinary bladder, we first examined whether there was a correlation between PTHrP mRNA level and the degree of bladder wall distension. Under normal conditions, PTHrP mRNA levels were much higher in more distended bladders than in less distended bladders. This observation suggested that PTHrP mRNA levels changed rather rapidly as a function of bladder distension in the physiological states. The results of the time course study further support this view by demonstrating a time-dependent increase in PTHrP mRNA expression in gradually distended bladders. Furthermore, immunohistochemical analysis confirmed that PTHrP was actually translated in smooth muscle cells of distended bladder.

The increase in PTHrP mRNA in the urinary bladder after accumulation of urine and stretching of the bladder wall may be caused by systemic and/or local factors. To determine whether the observed changes in PTHrP mRNA levels are mediated by systemic or local factor(s), we devised an *in vivo* model to limit the distension in either the proximal or distal half of the bladder without obstructing blood flow. When the levels of PTHrP mRNA were compared between distended and nondistended halves, they were found to be higher in the distended half. This observation indicates that PTHrP gene expression in the urinary bladder is most likely controlled by a local mechanism that is sensitive to stretching.

It is unlikely that intravesical pressure itself is a principal regulatory factor for PTHrP mRNA expression, as it is well established that under normal conditions the pressure inside the bladder remains stable over a wide range of bladder volume (28). In addition, the difference in PTHrP mRNA levels between distended and nondistended halves of the same bladder argues against the involvement of pressure in the regulation of PTHrP expression, because the intravesical pressure is equal within the same bladder even if part of the bladder is distended. Mechanical or hypoxic tissue injury is also an unlikely explanation for the increase in PTHrP mRNA during bladder distension. Significant increases in PTHrP mRNA levels were observed not only in markedly distended bladders but also in moderately distended bladders under physiological conditions. The possible involvement of neurotransmitters in the regulation of PTHrP mRNA expression has yet to be determined. We cannot completely rule out neural mechanisms since we did not perform denervation or pharmacological studies using neuroeffector blockers. However, the absence of micturition reflex during experiments under pentobarbital anesthesia and the findings in locally distended bladders preclude the systemic control of nervous system as a major regulator.

Thus it seems reasonable to conclude that the local mechanism(s) related to bladder wall stretching itself has a key role in controlling PTHrP gene expression in the urinary bladder. Several possibilities can be proposed as regulatory mechanisms. First, stretch- or cell-shape-mediated biological signals could regulate gene expression (29–31). Alternatively, local hemodynamic and metabolic changes that accompany the distension of the bladder (32) might stimulate PTHrP mRNA expression through unknown mediator(s).

It is a characteristic of the bladder that intravesical pressure is maintained at a relatively low level despite the

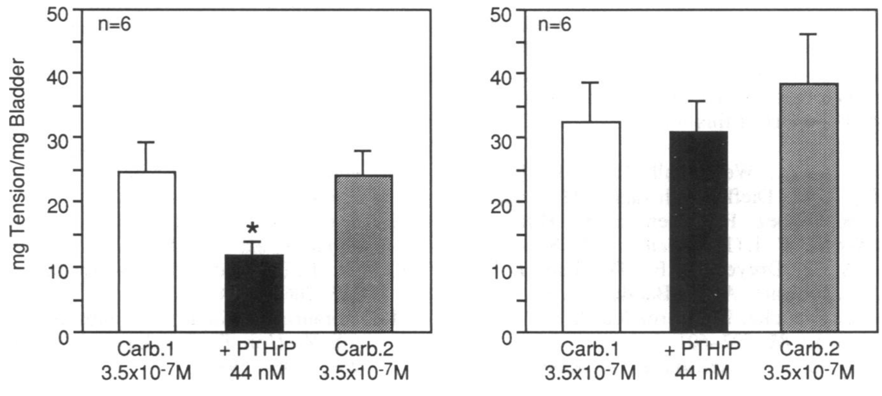

FIG. 5. Comparison of the muscle-relaxing effects of PTHrP on bladders kept empty and distended. Rats were treated in the same way as Fig. 2 to maintain the bladder empty (*Left*) or distended (*Right*) *in vivo*. Four hours later, bladders were removed and a muscle strip (≈15 × 2 mm) was cut from each bladder. An isolated strip was immediately placed in the organ bath and a 0.5-g resting tension was applied to each strip. Muscle contraction was induced by carbachol three times after a 30-min stabilization period for each challenge as follows: 1, carbachol (carb.1) alone (open bars); 2, carbachol plus PTHrP-(1–34)-NH_2 (solid bars); 3, carbachol (carb.2) alone (shaded bars).
*Significant difference from the results of carbachol alone ($P < 0.05$).

progressive increase in urine volume during the continence phase (28). This constant level of pressure is considered to be produced by continuous relaxation of the bladder smooth muscle. However, little is known about the regulatory mechanisms of the muscle relaxation. One of the possible mechanisms for the bladder muscle relaxation is inhibitory neural regulation of contraction (33). Another is an intrinsic myogenic regulation of muscle tone. In this context, PTHrP may be a candidate for the physiological muscle relaxant in the urinary bladder. We tested this possibility in the organ bath studies because it has been reported that exogenous PTHrP-(1–34) induces muscle relaxation in other smooth muscle tissues (18, 20–23).

The isolated organ bath studies showed that, in bladders maintained empty for 2 h *in vivo*, PTHrP-(1–34)-NH_2 relaxed the carbachol-induced contraction in a dose-dependent manner with EC_{50} value comparable to the values obtained in other tissues (20–23). An interesting finding in this study was that there was a difference in the ability of PTHrP-(1–34)-NH_2 to relax muscle strips isolated from bladders kept empty and distended *in vivo*. In bladders that had been kept empty for 4 h and were in a state of low PTHrP expression, PTHrP-(1–34)-NH_2 induced a significant muscle relaxation. On the other hand, the same dose of PTHrP-(1–34)-NH_2 failed to relax the muscle strips obtained from distended bladders that were in a state of high PTHrP expression. One possible and tempting explanation for the apparent refractoriness of the distended bladder to exogenous PTHrP-(1–34)-NH_2 may be that the high levels of endogenous PTHrP already relaxed the bladder muscle near maximally and desensitized its responsiveness to PTHrP. Similar PTHrP-specific desensitization for muscle relaxation has been reported to occur *in vitro* in the rat gastric smooth muscle (22).

The findings described in this study are consistent with the concept that the expression of the PTHrP gene in the urinary bladder is regulated by local changes in mechanical stretch. They also support a role for PTHrP as an autocrine/paracrine modulator of bladder smooth muscle relaxation. It is also possible that PTHrP may act in a paracrine fashion to relax vascular smooth muscle, since it has been shown that bladder distension is accompanied by an increase in blood flow to the tissue (32) and that PTHrP-(1–34) has vasorelaxant property (18, 20, 21). The present findings in the urinary bladder and the previous findings in the rat uterus (25) and the chicken oviduct vasculature (18) suggest that the synthesis by and the local action of PTHrP on smooth muscle may be a general physiological response of tissues that undergo continuous or periodic stretching and adjustment of muscle tonicity.

For his original support for the publication of this work, we dedicate this paper to the living memory of Dr. Gerald Aurbach. We thank R. M. Gardner and L. T. Duong for their assistance in this study, M. P. Caulfield for his critical review of the manuscript, and M. Rosenblatt for continued support of this work.

1. Suva, L. J., Winslow, G. A., Wettenhall, R. E. H., Hammonds, R. G., Moseley, J. M., Dieffenbach-Jagger, H., Rodda, C. P., Kemp, B. E., Rodriguez, H., Chen, E. Y., Hudson, P. J., Martin, T. J. & Wood, W. I. (1987) *Science* **237**, 893–896.
2. Mangin, M., Webb, A. C., Dreyer, B. E., Posillico, J. T., Ikeda, K., Weir, E. C., Stewart, A. F., Bander, N. H., Milstone, L., Barton, D. E., Francke, U. & Broadus, A. E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 597–601.
3. Thiede, M. A., Strewler, G. J., Nissenson, R. A., Rosenblatt, M. & Rodan, G. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4605–4609.
4. Broadus, A. E., Mangin, M., Ikeda, K., Insogna, K. L., Weir, E. C., Burtis, W. J. & Stewart, A. F. (1988) *N. Engl. J. Med.* **319**, 556–563.
5. Martin, T. J. & Suva, L. J. (1989) *Clin. Endocrinol.* **31**, 631–647.
6. Orloff, J. J., Wu, T. L. & Stewart, A. F. (1989) *Endocr. Rev.* **10**, 476–495.
7. Horiuchi, N., Caulfield, M. P., Fisher, J. E., Goldman, M. E., McKee, R. L., Reagan, J. E., Levy, J. J., Nutt, R. F., Rodan, S. B., Schofield, T. L., Clemens, T. L. & Rosenblatt, M. (1987) *Science* **238**, 1566–1568.
8. Kemp, B. E., Moseley, J. M., Rodda, C. P., Ebeling, P. R., Wettenhall, R. E. H., Stapleton, D., Diefenbach-Jagger, H., Ure, F., Michelangeli, V. P., Simmons, H. A., Raisz, L. G. & Martin, T. J. (1987) *Science* **238**, 1568–1570.
9. Thompson, D. D., Seedor, J. G., Fisher, J. E., Rosenblatt, M. & Rodan, G. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5673–5677.
10. Thiede, M. A. & Rodan, G. A. (1988) *Science* **242**, 278–280.
11. Ikeda, K., Weir, E. C., Mangin, M., Dannies, P. S., Kinder, B., Deftos, L. J., Brown, E. M. & Broadus, A. E. (1988) *Mol. Endocrinol.* **2**, 1230–1236.
12. Drucker, D. J., Asa, S. L., Henderson, J. & Goltzman, D. (1989) *Mol. Endocrinol.* **3**, 1589–1595.
13. Yasuda, T., Banville, D., Rabbani, S. A., Hendy, G. N. & Goltzman, D. (1989) *Mol. Endocrinol.* **3**, 518–525.
14. Weir, E. C., Brines, M. L., Ikeda, K., Burtis, W. J., Broadus, A. E. & Robbins, R. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 108–112.
15. Mangin, M., Ikeda, K. & Broadus, A. E. (1990) *Gene* **95**, 195–202.
16. Selvanayagam, P., Graves, K., Cooper, C. & Rajaraman, S. (1991) *Lab. Invest.* **64**, 713–717.
17. Schermer, D. T., Chan, S. D. H., Bruce, R., Nissenson, R. A., Wood, W. I. & Strewler, G. J. (1991) *J. Bone Miner. Res.* **6**, 149–155.
18. Thiede, M. A., Harm, S. C., McKee, R. L., Grasser, W. A., Duong, L. T. & Leach, R. M., Jr. (1991) *Endocrinology* **129**, 1958–1966.
19. Kramer, S., Reynolds, F. H., Jr., Castillo, M., Valenzuela, D. M., Thorikay, M. & Sorvillo, J. M. (1991) *Endocrinology* **128**, 1927–1937.
20. Winquist, R. J., Baskin, E. P. & Vlasuk, G. P. (1987) *Biochem. Biophys. Res. Commun.* **149**, 227–232.
21. Nichols, G. A., Nana, A. D., Nichols, M. A., DiPette, D. J. & Asimakis, G. K. (1989) *Endocrinology* **125**, 834–841.
22. Mok, L. L. S., Ajiwe, E., Martin, T. J., Thompson, J. C. & Cooper, C. W. (1989) *J. Bone Miner. Res.* **4**, 433–439.
23. Shew, R. L., Yee, J. A., Kliewer, D. B., Keflemariam, Y. J. & McNeill, D. L. (1991) *J. Bone Miner. Res.* **6**, 955–959.
24. Nichols, G. A., Nichols, M. A. & Helwig, J.-J. (1990) *Endocrinology* **126**, 721–727.
25. Thiede, M. A., Daifotis, A. G., Weir, E. C., Brines, M. L., Burtis, W. J., Ikeda, K., Dreyer, B. E., Garfield, R. E. & Broadus, A. E. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6969–6973.
26. Chorev, M., Goldman, M. E., McKee, R. L., Roubini, E., Levy, J. J., Gay, C. T., Reagan, J. E., Fisher, J. E., Caporale, L. H., Golub, E. E., Caulfield, M. P., Nutt, R. F. & Rosenblatt, M. (1990) *Biochemistry* **29**, 1580–1586.
27. Yamamoto, M., Duong, L. T., Fisher, J. E., Thiede, M. A., Caulfield, M. P. & Rosenblatt, M. (1991) *Endocrinology* **129**, 2614–2622.
28. Tanagho, E. A. (1988) in *Smith's General Urology*, eds. Tanagho, E. A. & McAninch, J. W. (Appleton & Lange, East Norwalk, CT), pp. 452–472.
29. Lansman, J. B., Hallam, T. J. & Rink, T. J. (1987) *Nature (London)* **325**, 811–813.
30. Gu, J., D'andrea, M. & Seethapathy, M. (1989) *Endocrinology* **125**, 2066–2074.
31. Komuro, I., Kaida, T., Shibazaki, Y., Kurabayashi, M., Katoh, Y., Hoh, E., Takaku, F. & Yazaki, Y. (1990) *J. Biol. Chem.* **265**, 3595–3598.
32. Andersson, P.-O., Bloom, S. R., Mattiasson, A. & Uvelius, B. (1985) *J. Urol.* **134**, 1041–1046.
33. Klarskov, P. (1988) *Neurourol. Urodyn.* **7**, 307–326.